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Tolerance of transformed cotton to glufosinate

Lesli K. Blair-Kerth
Department of Plant and Soil Science, Mailstop 2122, Texas Tech University, Lubbock, TX 79409-2122

Peter A. Dotray
Corresponding author. Department of Plant and Soil Science, Mailstop 2122, Texas Tech University, Lubbock, TX 79409-2122; p-dotray@tamu.edu

J. Wayne Keeling
John R. Gannaway
Texas Agricultural Experiment Station, Lubbock, TX 79401-9757

Mel J. Oliver
Jerry E. Quisenberry
Plant Stress and Water Conservation Laboratory, USDA-ARS, Lubbock, TX 79415

Field experiments from 1997 to 1999 examined cotton cv. 'Coker 312' that was genetically transformed to tolerate glufosinate. None of the glufosinate treatments caused visible injury to the glufosinate-tolerant cotton, but treatments were lethal to nontransformed or nonexpressing cotton. No glufosinate treatment adversely affected plant height at maturity, total number of nodes, bolls per plant, or boll positions. Glufosinate applications of 0.6 kg ha^{-1} made at eight stages of growth, ranging from cotyledon stage to 50% open boll, did not adversely affect yield or fiber quality as measured by micronaire or fiber length and strength. Sequential glufosinate applications up to four stages of growth from the zero- to one-leaf stage to the 14- to 15-leaf stage or individual glufosinate applications at 3.3 kg ha^{-1} made at the two- to three-leaf stage of growth also did not adversely affect yield or fiber quality. Overall yields in these studies were low relative to normal Texas Southern High Plains cotton yield because these studies were conducted using a Coker 312 parental line, which is generally a poor performer in this region. This research indicated that the transformation events for glufosinate tolerance in cotton were successful and the glufosinate-tolerance gene was expressed throughout the growing season. Transformation and field testing of other cotton varieties are needed to improve varietal performance on the Texas Southern High Plains.

Nomenclature: Glufosinate; cotton, *Gossypium hirsutum* L. 'Coker 312'.

Key words: Sequential applications, stage of growth, herbicide rate, bialaphos resistance gene, *BAR* gene.

Weed control programs in cotton traditionally are based on herbicides that are soil-applied, postemergence-directed (PD), or spot sprayed and on hand-hoeing and cultivation. However, weeds tolerant to soil-applied herbicides and weed escapes can adversely affect yield and quality and create harvest problems. Traditional postemergence herbicide programs in cotton may cause significant injury and yield loss if applied postemergence-topical (PT) and, therefore, must be applied PD (Snipes and Mueller 1992). Repeated herbicide applications are often needed to achieve desired control but may cause carryover problems to rotational crops (Knake 1992) and increase the development of herbicide-resistant weeds (Gressel and Segel 1990).

The use of herbicide-resistant cotton provides new opportunities to selectively control weeds in-season. Glyphosate-tolerant (Roundup Ready™) and bromoxynil-tolerant (BXN™) cotton are currently available to producers and provide improved PT management options to control weeds. Glyphosate-tolerant cotton allows producers to use glyphosate, a widely used nonselective herbicide, as a broadcast PT application. However, glyphosate cannot be sprayed PT in cotton after the four-true-leaf stage because of potential fruit abortion problems following applications just prior to or during reproductive development (Jones and Snipes 1999). Later applications of glyphosate must be made as a PD application (Brown 1997). With bromoxynil-tolerant cotton, bromoxynil can be applied PT from emergence to 75 d before harvest to control dicotyledonous weeds. However, bromoxynil provides unacceptable control of pigweed (*Amaranthus*) species, no control of grass weeds, and like glyphosate, lacks soil activity (Anonymous 2000).

Glufosinate tolerance has also been developed in rice (*Oryza sativa* L.) and tobacco (*Nicotiana tabacum* L.) and is commercially available in corn (*Zea mays* L.) and soybean (*Glycine max* L.) (Anonymous 1999). Glufosinate tolerance was achieved by insertion and expression of the bialaphos resistance gene (*BAR* gene) isolated from *Streptomyces hygroscopicus.* The *BAR* gene, which is responsible for coding for the phosphinothricin acetyl transferase (PAT) enzyme, detoxifies the L-isomer of glufosinate into an inactive acetylated derivative (Tsaftaris 1996).

In previous research (M. J. Oliver and J. E. Quisenberry, unpublished data) at the USDA-ARS facility in Lubbock, the *BAR* gene was introduced into cotton Coker 312 using *Agrobacterium*-mediated infection. Glufosinate-tolerant cotton Coker 312 was developed by the introduction of a chimeric *BAR* gene, driven by a cauliflower mosaic virus 35S promoter, utilizing the *Agrobacterium*-mediated transformation protocol described by Bayley et al. (1992). The chimeric *BAR* gene was constructed by inserting the *BAR* cod-

TABLE 1. Glufosinate-tolerant cotton (*Gossypium hirsutum*) stage of growth and glufosinate application dates in single application tolerance experiments in 1997 and 1998.

	Date of application	
Stage of growth	1997	1998
Cotyledon	June 13	June 8
2–3 leaves	June 23	June 18
4–5 leaves	July 1	June 25
First square	July 15	July 9
First bloom	July 28	July 27
Peak bloom	Aug 11	Aug 7
Cut-out	Sept 5	Aug 26
50% open boll	Oct 5	Oct 1
Nontreated	—	—

Table 2. Stage of growth of cotton (*Gossypium hirsutum*) and dates of glufosinate applications in sequential application stage of growth tolerance experiments.

Number of mainstem leaves at application	Date of application		
	1997	1998	1999
0–1	June 13	June 8	May 28
3–4	June 27	June 25	June 9
9–10	July 15	July 9	June 30
14–15	July 28	July 27	July 19
0–1, 3–4	June 13, 27	June 8, 25	May 28, June 9
0–1, 9–10	June 13, July 15	June 8, July 9	May 28, June 30
0–1, 14–15	June 13, July 28	June 8, July 27	May 28, July 19
3–4, 9–10	June 27, July 15	June 25, July 9	June 9, 30
3–4, 14–15	June 27, July 28	June 25, July 27	June 9, July 19
9–10, 14–15	July 15, 28	July 9, 27	June 30, July 19
0–1, 3–4, 9–10	June 13, 27; July 15	June 8, 25; July 9	May 28; June 9, 30
0–1, 3–4, 14–15	June 13, 27; July 28	June 8, 25; July 27	May 28, June 9, July 19
3–4, 9–10, 14–15	June 27; July 15, 28	June 25; July 9, 27	June 9, 30; July 19
0–1, 3–4, 9–10, 14–15	June 13, 27; July 15, 28	June 8, 25; July 9, 27	May 28; June 9, 30; July 19
Nontreated	—	—	—

ing sequence from pAHC25 (Christensen and Quail 1996) into pRTL2 (Li and Carrington 1995), replacing the tobacco mosaic virus (TMV) leader sequence and placing the *BAR* coding sequence between the 35S promoter and 355 termination signal sequence. The complete chimeric gene was subsequently removed from pRTL2 as a *Hin*dIII fragment and cloned into the multicloning site of pBIN19 (Bevan 1984) for transfer into the *Agrobacterium tumefaciens* strain EHA105 (Hood et al. 1993). Infected plants were screened for tolerance in greenhouse experiments by applying a 2% glufosinate solution. The objectives of this research were to evaluate growth, development, and yield in field-grown glufosinate-tolerant cotton following glufosinate applications made at various stages of growth, at various rates, and with sequential applications.

Materials and Methods

Field experiments were conducted from 1997 to 1999 at the Texas Agricultural Experiment Station near Lubbock, TX, to determine the tolerance of transformed cotton to glufosinate. The soil type was an Acuff sandy clay loam (fine-loamy, mixed, thermic Aridic Paleustoll), pH 7.8, and 0.7% organic matter. The glufosinate-tolerant cotton parental seed line used was Coker 312. Cotton seed was treated with captan {*N*-[(trichloromethyl)thio]-4-cyclohexene-1,2-dicarboximide} at 1.3 ml kg^{-1} seed, myclobutanil [α-butyl-α-(4-chlorophenyl)-1*H*–1,2,4-triazole-1-propanenitrile] at 0.8 ml kg^{-1} seed, and metalaxyl [*N*-(2,6-dimethylphenyl)-*N*-(methoxyacetyl)-alanine methyl ester] at 0.5 ml kg^{-1} seed prior to planting. Traditional production practices were used to maintain cotton development, growth, and yield. Nitrogen at 67.2 kg ha^{-1} and phosphorus at 44.8 kg ha^{-1} were applied according to soil testing recommendations. Glufosinate-tolerant cotton was planted at 10.4 kg ha^{-1} in 102-cm rows on May 31, 1997. Plot size was 4 by 8 m, but only the center two rows were planted. Because of increased seed availability, 22.4 kg ha^{-1} were planted on May 25, 1998, and May 13, 1999, in the entire 4- by 12-m plots.

A single glufosinate application stage of growth tolerance test and a sequential glufosinate application stage of growth tolerance test were conducted in 1997 and 1998, whereas the glufosinate application rate tolerance test was conducted in 1997, 1998, and 1999. For the single application stage

Table 3. Rainfall distribution for the Texas Southern High Plains by month from 1997 to 1999 compared to the 30-yr average.

Month	Rainfall			
	1997	1998	1999	30-yr avg.
	mm			
Jan	60	0	36	13
Feb	34	50	91	16
March	0	37	27	20
April	145	8	54	30
May	68	0	110	65
June	68	38	101	64
July	45	0	25	56
Aug	38	91	15	51
Sept	40	0	83	64
Oct	35	62	19	51
Nov	16	20	0	16
Dec	4	15	0	16
Total	500	320	561	460

Table 4. Effects of a single glufosinate application at various stages of growth on cotton (*Gossypium hirsutum*) lint yield and HVI[a] measurements averaged across 1997 and 1998.

Growth stage	Yield	Fiber properties		
		Micronaire	Length	Strength
	kg ha^{-1}	units	mm	kN m kg^{-1}
Cotyledon	632	4.5	29	294
2–3 leaves	611	4.3	29	294
4–5 leaves	604	4.0	29	294
First square	647	3.9	29	294
First bloom	608	4.0	29	294
Peak bloom	595	3.8	29	294
Cut-out	651	4.1	29	314
50% open boll	568	4.2	30	294
Nontreated	640	4.3	29	294
Standard error	45.9	0.16	0.51	6.6
LSD (0.05)	NS	NS	NS	NS

[a] High volume instrument classing.

TABLE 5. Effects of a single glufosinate application at various stages of growth on the growth and development of cotton (*Gossypium hirsutum*) averaged across 1997 and 1998.

Stage of growth	Height	Total nodes	Bolls	Bolls by position[a]			FP1 retention by mainstem node	
				FP1	FP2	FP > 2	6–10	11–15
	m		plant^{-1}	%				
Cotyledon	0.45	15.4	10.3	62	28	9	75	43
2–3 leaves	0.41	15.0	10.3	62	29	8	78	37
4–5 leaves	0.43	15.5	9.6	60	27	12	74	31
First square	0.42	14.8	9.6	63	28	6	75	33
First bloom	0.42	14.9	9.6	60	31	9	69	44
Peak bloom	0.44	15.2	9.2	63	28	6	75	29
Cut-out	0.42	15.1	9.9	63	26	9	77	35
50% open boll	0.43	14.7	9.2	68	24	6	76	34
Nontreated	0.43	15.1	9.7	61	29	9	73	36
Standard error	0.01	0.87	0.43	0.76	3.15	2.31	2.38	4.12
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS

[a] FP1, fruiting position 1; FP2, fruiting position 2; FP > 2, fruiting positions greater than 2.

of growth tolerance test, glufosinate was applied at 0.6 kg ha^{-1} to glufosinate-tolerant cotton from cotyledon to 50% open boll stage of growth (Table 1). For the sequential application stage of growth tolerance test, glufosinate at 0.6 kg ha^{-1} was applied from the 0 to 1 to the 14 to 15 stages of growth (Table 2). For the application rate tolerance test, glufosinate was applied at 0.4, 0.8, 1.6, and 3.3 kg ha^{-1} to glufosinate-tolerant cotton at the two- to three-leaf stage on June 23, 1997, and June 18, 1998. No irrigation was applied in 1997 because of timely and adequate rainfall. However, 25 cm of supplemental furrow irrigation was applied during the 1998 and 1999 growing seasons because of below-average rainfall (Table 3). Herbicide applications were applied PT to the entire two-row plots in 1997 and to the middle two rows of each four-row plot in 1998 and 1999.

TABLE 6. Effects of a single application or sequential applications of glufosinate on cotton (*Gossypium hirsutum*) yield and HVI[a] measurements averaged across 1997, 1998, and 1999.

Number of mainstem leaves	Yield	Fiber properties		
		Micronaire	Length	Strength
	kg ha^{-1}	units	mm	kN m kg^{-1}
0–1	527	4.3	29	284
3–4	540	4.3	29	294
9–10	596	4.3	29	294
14–15	530	4.2	28	294
0–1, 3–4	515	4.2	28	284
0–1, 9–10	614	4.2	29	294
0–1, 14–15	544	4.1	29	294
3–4, 9–10	627	4.3	29	294
3–4, 14–15	555	4.2	28	294
9–10, 14–15	588	4.3	29	294
0–1, 3–4, 9–10	587	4.1	29	304
0–1, 3–4, 14–15	577	4.4	29	294
3–4, 9–10, 14–15	556	4.2	29	294
0–1, 3–4, 9–10, 14–15	547	4.3	29	284
Nontreated	594	4.3	29	294
Standard error	39.4	0.08	0.25	4.5
LSD (0.05)	NS	NS	NS	NS

[a] High-volume instrument classing.

Applications were made using either a tractor-mounted compressed-air sprayer or a CO_2 backpack sprayer equipped with 80015VS spray nozzles calibrated to deliver 71 L ha^{-1} aqueous solution at 207 kPa. Plots were cultivated twice and hand-weeded throughout the growing season to maintain weed-free conditions.

Cotton stand in one row by 2 m was recorded 7 d after treatment (DAT) to determine segregation of nontolerant plants. Visual injury was evaluated 7, 14, and 21 DAT using a 0 (no visual injury) to 100% (complete necrosis) scale. Heights of five randomly selected plants per plot were recorded 21 and 54 DAT. At harvest, cotton was plant-mapped to determine plant height; number of mainstem nodes; and number of bolls, bolls by position, and retention of first-position bolls according to the method described by Hake et al. (1996). Yield was determined by hand-harvesting 2 by 2 m within each plot. In 1997, no cotton row borders existed on the 2-row plots because of limited seed. However, blank rows were present as borders between plots. In 1998 and 1999, the center two rows of the four-row plots were harvested. Fiber quality (length, strength, and micronaire) was analyzed with the use of classing by high-volume instruments (HVI) at the International Textile Center (Texas Tech University, Lubbock, TX).

For each tolerance test, the experimental design was a randomized block design with three replications. Data were subjected to an analysis of variance and means were separated using Fisher's Protected LSD test at the 5% probability level. No herbicide treatment by year interaction was observed in any of the tolerance experiments; therefore, data in each test were combined over years. Homogeneity of variances were tested using Levene's Test for constant variance. All variances were homogeneous. Therefore, no transformations were necessary.

Results and Discussion

In 1997, 11% of the cotton planted in each study was not tolerant to glufosinate. These plants became chlorotic and necrotic within a few days after the single application or the first application in sequential treatments. This symptomatology is similar to glufosinate applied to susceptible

TABLE 7. Effects of single application or sequential applications of glufosinate on growth and development of cotton (*Gossypium hirsutum*) averaged across 1997, 1998, and 1999.

Number of mainstem leaves at application	Height	Total nodes	Bolls	Bolls by position[a]			FP1 retention by mainstem node	
				FP1	FP2	FP > 2	6–10	11–15
	m		plant^{-1}	%				
0–1	0.43	15.2	9.5	62	26	14	68	37
3–4	0.41	15.6	10.1	60	26	10	69	40
9–10	0.43	15.3	10.6	60	28	12	72	37
14–15	0.41	15.1	8.7	65	27	8	76	29
0–1, 3–4	0.42	15.2	9.2	66	27	8	73	32
0–1, 9–10	0.44	15.6	10.0	57	27	11	69	37
0–1, 14–15	0.42	15.2	9.4	66	24	7	78	36
3–4, 9–10	0.43	15.5	10.0	60	28	11	76	32
3–4, 14–15	0.43	15.4	10.4	59	29	13	75	39
9–10, 14–15	0.41	15.0	10.2	63	26	11	80	39
0–1, 3–4, 9–10	0.41	15.0	9.9	59	27	15	72	33
0–1, 3–4, 14–15	0.44	15.4	10.2	58	29	10	72	35
3–4, 9–10, 14–15	0.43	15.4	9.8	61	27	9	73	35
0–1, 3–4, 9–10, 14–15	0.41	15.4	9.4	63	27	7	73	39
Nontreated	0.42	15.0	9.5	61	28	9	66	37
Standard error	0.01	0.50	0.26	0.65	2.87	1.80	7.53	6.53
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS

[a] FP1, fruiting position 1; FP2, fruiting position 2; FP > 2, fruiting positions greater than 2.

plants or nontransformed cotton. The number of susceptible plants decreased to less than 2% in the 1998 and 1999 field tests because seed used in 1998 and 1999 was collected from tolerant plants in 1997. Plants not tolerant to glufosinate may not contain the *BAR* gene or the gene may be present but is not expressed at sufficient levels.

Weather conditions were recorded for all glufosinate applications (data not shown). The conditions at application (temperature and relative humidity) were not constant in this semiarid environment. However, cotton tolerance to glufosinate did not change.

Single Glufosinate Application Stage of Growth Tolerance Test

No visual injury or reduction in plant height was observed following a single application of glufosinate applied at various stages of growth (data not shown). Glufosinate applications made at eight stages of growth, ranging from cotyledon stage to 50% open boll, did not adversely affect yield or fiber quality as measured by micronaire or fiber length and strength ($P > 0.05$) (Table 4). This contrasts research with glyphosate-tolerant cotton, where applications after the four-node stage of growth resulted in yield reductions (Baughman et al. 1999; Kalaher et al. 1997).

Glufosinate had no effect on plant height at harvest; total nodes; average number of bolls per plant; percentage of bolls by fruiting position one, two, or greater than two; and percentage of fruiting position one retention on mainstem nodes 6 to 10 and 11 to 15 ($P > 0.05$) (Table 5). In contrast, Kalaher et al. (1997) found that with glyphosate-tolerant cotton, applications at the eight-leaf stage and at the first white bloom stage resulted in a lower number of first and second bolls at nodes 4 to 7. Moreover, Ferreira (1998) and Reynolds et al. (1999) reported that nonlabeled PT applications of glyphosate at the 10- and 14-node stage and the 6- to 12-node stage, respectively, caused lower fruit retention. However, Reynolds reported that lower fruit retention did not affect yields but delayed crop maturity.

TABLE 8. Effects of application rate of glufosinate on cotton (*Gossypium hirsutum*) yield and HVI[a] measurements averaged across 1997 and 1998.

Glufosinate rate[b]	Yield	Fiber properties		
		Micronaire	Length	Strength
kg ai ha^{-1}	kg ha^{-1}	units	mm	kN m kg^{-1}
0.41	615	4.3	29	304
0.82	616	4.6	29	294
1.64	541	4.4	29	294
3.27	595	4.3	29	304
Nontreated	671	4.4	29	294
Standard error	53.85	0.11	0.25	7.6
LSD (0.05)	NS	NS	NS	NS

[a] High-volume instrument classing.
[b] Glufosinate applied to cotton at the two- to three-leaf stage of growth.

Sequential Glufosinate Application Stage of Growth Tolerance Test

No visual injury was observed 7, 14, or 21 DAT (data not shown). No reduction in plant height was observed 21 and 56 DAT (data not shown). Lint yield, micronaire, length, and strength were not affected by any glufosinate application (Table 6). At harvest, plant height; total nodes per plant; average number of bolls per plant; percentage of bolls at fruiting position one, two, or greater than two; and percent retention of fruiting position on mainstem nodes 6 to 10 and 11 to 15 were measured and shown in Table 7. No single or sequential glufosinate application adversely affected any of the plant mapping parameters collected. This was similar to reports by Jones et al. (1994), who found no

TABLE 9. Effects of application rate of glufosinate on growth and development of cotton (*Gossypium hirsutum*) averaged across 1997 and 1998.

Glufosinate rate[a]	Height	Total nodes	Bolls	Bolls by position[b]			FP1 retention by mainstem node	
				FP1	FP2	FP > 2	6–10	11–15
kg ha^{-1}	m			%				
0.41	0.45	15.2	9.4	66	23	8	80	33
0.82	0.43	15.5	9.9	64	26	8	79	40
1.64	0.48	16.3	10.0	63	25	12	64	53
3.27	0.47	15.6	10.2	66	27	7	81	43
Nontreated	0.48	15.5	11.3	56	28	11	79	39
Standard error	0.01	0.80	0.36	0.79	3.69	2.42	2.38	4.37
LSD (0.05)	NS	NS	NS	NS	NS	NS	NS	NS

[a] Glufosinate applied to cotton at the two- to three-leaf stage of growth.
[b] FP1, fruiting position 1; FP2, fruiting position 2; FP > 2, fruiting positions greater than 2.

significant differences in yield or fiber quality when bromoxynil was applied PT to bromoxynil-tolerant cotton with six to eight true leaves followed by applications 14 d later. Matthews et al. (1997) found that percent first harvest in glyphosate-tolerant cotton was reduced by 4% when glyphosate was applied at the 8- or 10-leaf stage following a four-leaf stage treatment; however, lint yields did not differ in any treatment.

Glufosinate Application Rate Tolerance Test

No visual injury or plant height reductions were observed following any rate of application of glufosinate (data not shown). No adverse effects in yield, micronaire, length, strength, leaf grade, or color grade were found regardless of the glufosinate rate (Table 8). At harvest, plant height; total plant nodes; average number of bolls per plant; percentage of bolls by fruiting position one, two, or greater than two; and percentage of fruiting position one retention on mainstem nodes 6 to 10 and 11 to 15 were not affected ($P > 0.05$) by any glufosinate rate (Table 9). Similar results were observed in bromoxynil-tolerant cotton, where no crop injury was observed following bromoxynil applications up to 81.7 kg ha^{-1} (Collins 1996). When glyphosate was applied at 1.7 kg ae ha^{-1} to glyphosate-tolerant cotton at node 6 and node 9, yields were reduced (Brown and Bednarz 1998).

These studies indicated that yield; micronaire; length; strength; plant height; total nodes per plant; average number of bolls per plant; percentage of bolls by fruiting position one, two, or higher; and percentage of fruiting position one retention on mainstem nodes 6 to 10 and 11 to 15 were not affected by glufosinate regardless of the rate, stage of growth at application, or number of applications. Although overall yields in these studies were low relative to normal High Plains cotton yield, these studies were conducted using a Coker 312 parental line, which is generally a poor performer in this region. This study indicates that regionally adapted glufosinate-tolerant cotton cultivars can be developed for improved weed management systems based on glufosinate without tolerance limitations.

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